Structure–Activity Relationships of Ethylenimines. II. Methyl-Substituted Derivatives of Tetramin[1]

ALBERT T. BOTTINI AND VASU DEV

Chemistry Department, University of California, Davis, California

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The products from the reactions of butadiene monoxide (III) with 2-methylaziridine (IIb) and 2,2-dimethylaziridine (IIc) were found by n.m.r. spectroscopy to consist of about 70% of the 1-(1-aziridinyl)-3-buten-2-ol (I) and 30% of the corresponding 2-(1-aziridinyl)-3-buten-2-ol. The relative antineoplastic activities of the mixtures of aminobutenols and the mixtures obtained from III and aziridine (IIa), D-IIb, and L-IIb are discussed.

We reported recently that 1-(2-methylene-1-aziridinyl)-3-buten-2-ol (Id), the allenimine analog of 1-(1-aziridinyl)-3-buten-2-ol (Ia), is less active than Tetramin[2,3] against the mouse tumors Adenocarcinoma 755, Leukemia L-1210, and Sarcoma 180.[5] It was pointed out that the greater bulk of the exocyclic methylene group as compared with that of two hydrogens may cause a steric interaction with a group near the site of biological activity which impedes the proper alignment of Id in the biological system necessary for antineoplastic activity. It appeared that this possibility could be tested by preparing 1-(2-methyl-1-aziridinyl)-3-buten-2-ol (Ib) and 1-(2,2-dimethyl-1-aziridinyl)-3-buten-2-ol (Ic) and determining their activity against mouse tumors.

In order to prepare Ib and Ic, butadiene monoxide (III) was treated with 2-methylaziridine (IIb) and 2,2-dimethylaziridine (IIc), respectively, in the manner described for the preparation of Tetramin.[4]

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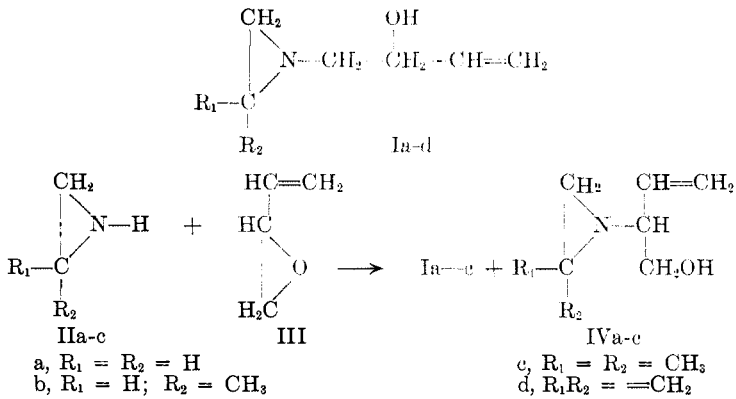
(2) (a) For a summary of the clinical data on Tetramin, compiled by F. R. White, see *Cancer Chemotherapy Reports*, **4**, 52 (1959), Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md. (b) See also J. M. Venditti, A. Goldin, and I. Kline, *ibid.*, **11**, 73 (1961).

(3) Tetramin, the product from the reaction of aziridine and butadiene monoxide, was assigned structure Ia.[4] It has been found recently that Tetramin is a 2:1 mixture of Ia and 2-(1-aziridinyl)-3-buten-1-ol (IVa). The isomers have been separated and characterized by nuclear magnetic resonance (n.m.r.) spectroscopy. (Private communication from E. M. Chamberlin.)

(4) K. Vierling, H. Öttel, and G. Wilhelm, German Patent 1,004,614; *Chem. Abs.*, **52**, 10,201 (1958).

(5) Part I: A. T. Bottini and V. Dev, *J. Org. Chem.*, **27**, 968 (1962).



The products had the correct elemental analyses for Ib and Ic, and their infrared spectra each contained bands at 3320 cm.$^{-1}$, characteristic of oxygen–hydrogen bonds,[6a] and 1660 cm.$^{-1}$, characteristic of carbon–carbon double bonds.[6b] However, examination of the n.m.r. spectra of the products, which was made after antitumor tests had begun, indicated that they were mixtures of isomers. The most noteworthy feature of each of the rather complicated n.m.r. spectra was a doublet of moderate intensity at $\tau = 6.55$,[7] $J = 5.7$–6.0. This band, also in the spectrum of Tetramin (V),[3,4] which was prepared for purposes of comparison, could not be explained as due to the 1-(1-aziridinyl)-3-buten-2-ol (I) structure.

The spectrum of essentially pure (−)-Id contained no band near $\tau = 6.55$, but the spectrum of (±)-Id, the method of preparation of which did not preclude contamination with the isomeric 2-(2-methylene-1-aziridinyl)-3-buten-1-ol (IVd, $R_1R_2 = =CH_2$), did contain a weak doublet at $\tau = 6.60$, $J = 6.2$.[5] As the spurious band present in the spectrum of (±)-Id was explained satisfactorily as due to the C_1-hydrogens of IVd, the bands at $\tau = 6.55$ present in the spectra of the products from the reactions of butadiene monoxide (III) with IIa, IIb, and IIc (hereafter referred to as Tetramin (V), "methyltetramin" (VI), and "dimethyltetramin" (VII), respectively) were assigned to the C_1-hydrogens of the corresponding 2-(1-aziridinyl)-3-buten-1-ol (IVa-c, respectively).

Methyltetramin and dimethyltetramin were distilled through a column of moderate efficiency. Examination of the fractions confirmed that each product was a mixture of secondary alcohol and primary

(6) (a) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1958, p. 96; (b) p. 34.

(7) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958). τ-Values reported here are considered accurate to $\pm 0.05\tau$.

alcohol, and that the bands at $\tau = 6.55$ were due to the higher boiling primary alcohols. Fractionation of VI and VII, and analogy with the n.m.r. spectra of other aminobutenols, simplified the interpretation of their n.m.r. spectra. The spectra of VI and VII, which are in agreement with spectra expected of mixtures of I and IV, are described in detail in the Experimental Section.

From the relative areas of the various bands in their n.m.r. spectra, the composition of methyltetramin (VI) was estimated as 69% 1-(2-methyl-1-aziridinyl)-3-buten-2-ol (Ib) and 31% 2-(2-methyl-1-aziridinyl)-3-buten-1-ol (IVb), and the composition of dimethyltetramin (VII) was estimated as 74% 1-(2,2-dimethyl-1-aziridinyl)-3-buten-2-ol (Ic) and 26% 2-(2,2-dimethyl-1-aziridinyl)-3-buten-1-ol (IVc). The estimates, which are considered accurate to $\pm 5\%$, were made assuming that the areas of the various bands were directly proportional to the number of hydrogens, and that the only components were the products formed by attack of the imine on the epoxide carbons, *i.e.*, I and IV. The presence of weak bands at $\tau = 5.9$ and 7.1 in the spectrum of the highest boiling fraction of methyltetramin could be due to the allylic hydrogens of 1-(1-aziridinyl)-2-buten-4-ol (VIII), the product that would be formed by S_N2' attack of the aziridine on butadiene monoxide (III). If these bands are due to VIII rather than some minor impurity, it could be estimated that VIII accounted for no more than 4% of the product formed by reaction of 2-methylaziridine with III. The reaction of 2,2-dimethylaziridine with III appeared to give less than 2%, if any, of the product of an S_N2' reaction. It is noteworthy that the reaction of diethylamine with III does not yield a detectable amount ($\sim 3\%$) of the product of an S_N2' reaction.[5,8]

There appears to be considerable variation in the ratio of the products formed by attack of different amines on the primary carbon and the secondary allylic carbon of III, and the relative amount of N,N-disubstituted-1-amino-3-buten-2-ol, formed by attack of a secondary amine at the primary carbon, appears to increase as the base strength of the attacking amine increases *and* as the steric requirements of the attacking amine increases. Thus, the secondary amines IIb, IIc, and diethylamine yield mixtures containing 69%, 74%, and 93% of the 1-amino-3-buten-2-ol, respectively.[9] The relative importance of electronic effects and steric effects in ring-opening

(8) F. F. Blicke and J. H. Biel, *J. Am. Chem. Soc.*, **79**, 5508 (1957).

(9) See R. M. Laird and R. E. Parker, *ibid.*, **83**, 4277 (1961), and J. K. Addy, R. M. Laird, and R. E. Parker, *J. Chem. Soc.*, 1708 (1961), for results of studies on the role of electronic factors in the reactions of benzylamine with phenyl-substituted styrene oxides.

reactions of butadiene monoxide with amines remains to be determined.

The results of antitumor tests with methyltetramin (VI) and dimethyltetramin (VII), which became available before we were aware that VI and VII were mixtures of primary and secondary alcohols, prompted us to prepare the optically active diastereoisomeric mixtures of VI in which the absolute configurations about the 2-aziridinyl carbon were known. These were obtained by treatment of III with D- and L-IIb.[10] D- and L-IIb were prepared from D- and L-alanine, respectively, by a four-step sequence[11] in which none of the bonds to the asymmetric center was broken. The product mixtures from the reactions of III with D- and L-IIb were levorotatory and dextrorotatory, respectively. Although the conditions used for preparing D-(−)-VI and L-(+)-VI were not the same as those for preparing the racemic VI, the relative amounts of Ib and IVb in the three mixtures appeared to be identical.

Because of the small quantities of D-(−)-VI and L-(+)-VI available to us after submittal of samples for testing, no attempts were made to separate either mixture into its components. It should be noted that treatment of either D-IIb or L-IIb with optically pure butadiene monoxide should give a mixture, separable by distillation, of only two of the eight diastereoisomers of Ib and IVb. Unfortunately, there appears to be no experimentally feasible route to optically pure butadiene monoxide.[5]

Antineoplastic Activity.[12]—At doses of 15 and 30 mg./kg./day, methyltetramin (VI) had 0.35 and 0.39 times the activity reported for smaller doses (7.5 mg./kg./day) of Tetramin (V) against the mouse tumor Leukemia L-1210,[2] and dimethyltetramin (VII), at doses of 15 mg./kg./day, had 0.20 times the activity reported for V. Against the mouse tumor Sarcoma 180, at doses of 20 mg./kg./day, VI and VII were 0.98 and 0.50 times as active, respectively, as V[2] at doses of 10 mg./kg./day. Against the mouse tumor Adenocarcinoma 755, at doses of 15 mg./kg./day, L-(+)-VI, VI, D-(−)VI, and VII were 0.93, 0.78, 0.38, and 0.25 times as active, respectively, as V[2] at doses of 3.75 mg./kg./day. Also against Adenocarcinoma 755,

(10) Using the systematic specification of absolute configuration proposed by R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956), D- and L-IIb are R- and S-IIb, respectively.

(11) Y. Minoura, M. Takebayashi, and C. C. Price, *J. Am. Chem. Soc.*, **81**, 4689 (1959).

(12) Samples of the new methyl-substituted derivatives of Tetramin were submitted to the Cancer Chemotherapy National Service Center (CCNSC), and antitumor tests were carried out at the Stanford Research Institute under the auspices of the CCNSC. The opinions expressed in this paper are those of the authors and not necessarily those of the CCNSC. Test data received from the CCNSC may be obtained from the authors on request.

but at doses of 7.5 mg./kg./day, L-(+)-VI and D-(−)-VI were 0.39 and 0.17 times as active as V.[2]

The results with Tetramin (V), methyltetramin (VI), and dimethyltetramin (VII) can be interpreted as indicating that the antineoplastic activity of mixtures of 1-(1-aziridinyl)-3-buten-2-ols (I) and 2-(aziridinyl-3-buten-1-ols (IV), at least against Leukemia L-1210, Sarcoma 180, and Adenocarcinoma 755, is reduced as the size of the groups attached to the aziridine ring is increased. This interpretation, if proved correct, would be in accord with the proposition that an increase in bulk of substituents on the aziridine ring impedes the alignment of the aziridinyl-butenols within the biological system necessary for antineoplastic activity to occur. However, it must be pointed out that the activities noted for V, VI, and VII are not necessarily equal to the sums of the activities of the individual components, because components of the mixtures could conceivably potentiate or diminish the activity of each other.[12a] Therefore, the apparent correlation of structure with antineoplastic activity of mixtures of I and IV must be accepted as tentative until test results on the pure components are available.

Although the greater activity of L-(+)-VI against Adenocarcinoma 755 is suggestive of a relationship between biological activity and the absolute configuration of a substituted carbon on the aziridine ring, such an interpretation is tenuous, again because of the possibilities that components of the mixtures could cause enhancement or diminution of the activity of each other. The existence or non-existence of a relationship between biological activity and the absolute configuration of a substituted carbon on the aziridine ring must await the results of tests now in progress with pure, optically active, alkyl-substituted derivatives of proved antineoplastic agents which have been prepared in this laboratory.

Experimental

Boiling points are uncorrected. Infrared spectra were obtained using a Beckman IR-4 Spectrophotometer. N.m.r. spectra were obtained using a Varian Associates HR-60 system with samples in 5-mm. o.d. tubes, and resonance frequencies were determined relative to the internal standard tetramethylsilane using the "sideband technique" with a Hewlett-Packard 200 CD audiooscillator. Microanalyses were performed by Mr. V. H. Tashinian, Berkeley, California.

Methyltetramin (VI).—To a cold (15°), stirred solution prepared from 39.2 g. (0.688 mole) of 2-methylaziridine (IIb, Monomer-Polymer Corp.) and 6.2 ml. of

(12a) The activity of Ia against Leukemia L-1210 is not affected by the presence of much less active IVa, and the toxicity of IVa does not appear to be affected by the presence of much less toxic Ia.[2b]

water was added dropwise 24.1 g. (0.344 mole) of butadiene monoxide (III)[5] in 20 min. Stirring was continued for 2 hr., and the mixture was allowed to stand at room temperature for 16 hr. The unreacted imine and most of the solvent were removed by distillation at 30 mm. until the head temperature reached 50°. The residue was distilled, and 24.1 g. (55%) of colorless product was collected at 60–63° (1.5 mm.), $n^{24}D$ 1.4619. The product was found by n.m.r. spectroscopy to consist of 69% 1-(2-methyl-1-aziridinyl)-3-buten-2-ol (Ib) and 31% 2-(2-methyl-1-aziridinyl)-3-buten-1-ol (IVb).

Anal. Calcd. for $C_7H_{13}NO$: C, 66.10; H, 10.30; N, 11.02. Found: C, 65.87; H, 10.47; N, 11.04.

Methyltetramin (14.0 g.) was distilled through a 60-cm. Podbielniak-type column.[13] The first fraction (3.7 g.) had b.p. 83.5–84° (12.4 mm.), $n^{24}D$ 1.4611, and examination of its n.m.r. spectrum indicated that it was composed of 95% IIb and 5% IVb. The pot residue (1.1 g.) was distilled through a semimicro Vigreux column at 77–78° (3.4 mm.). This fraction, which had $n^{23}D$ 1.4640, consisted of approximately 85–90% of IVb and less than 5% of Ib. If a band at $\tau = 7.1$ in the n.m.r. spectrum of the fraction is due solely to the C_1-hydrogens of VIII, the fraction contains 8% VIII.

Dimethyltetramin (VIII) was prepared in essentially the same manner as described for VI using 8.5 g. (0.121 mole) of 2,2-dimethylaziridine (IIc, Monomer–Polymer Corp.) and 2.2 g. of water. The product, which was found by n.m.r. spectroscopy to consist of 74% 1-(2,2-dimethyl-1-aziridinyl)-3-buten-2-ol (Ic) and 26% 2-(2,2-dimethyl-1-aziridinyl-3-buten-1-ol (IVc), weighed 7.5 g. (44%) and had b.p. 60–65° (1 mm.), $n^{24}D$ 1.4578.

Anal. Calcd. for $C_8H_{15}NO$: C, 68.04; H, 10.71; N, 9.92. Found: C, 67.61; H, 11.06; N, 9.88.

Dimethyltetramin (14 g.) was distilled through a Podbielniak-type column. The first fraction (2.0 g.), b.p. 87–89° (15 mm.), consisted of approximately 90% Ic and 10% IVc. The pot residue was distilled through a semimicro Vigreux column. It appeared to consist solely of Ic and IVc in a ratio of 1:2.

D- and L-2-Methylaziridine (IIb).—D-Alanine (50 g.) and L-alanine (100 g.) obtained from Nutritional Biochemicals Corp., were converted by the method of Fischer[14] to D-alanine ethyl ester, b.p. 65–66° (33 mm.), $n^{30}D$ 1.4139, $[\alpha]^{26}D$ − 3.2° (0.186 g./10 ml. in dry benzene), and L-alanine ethyl ester, b.p. 48–50° (11 mm.), $n^{30}D$ 1.4139, in yields of 38% and 45%, respectively. Alanine ethyl ester is reported[11] to have b.p. 60–61° (24 mm.), $n^{26}D$ 1.4204. Ethanol solutions of D-alanine ethyl ester (0.293 g./10 ml.) and its enantiomorph (0.234 g./10 ml.) were observed to have virtually no optical activity ($[\alpha]^{25}D \pm 0.2°$) within 1 hr. after preparation.[15]

Using the methods described by Minoura, Takebayashi, and Price,[11] the esters were reduced in comparable yields with lithium aluminum hydride to the corresponding alaninols, and the alaninols were converted with sulfuric acid to the 2-aminopropyl sulfuric acids in essentially quantitative yields. Treatment of 20.0 g. (0.13 mole) of D-2-aminopropyl sulfuric acid with sodium hydroxide solu-

(13) J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry," Prentice-Hall Inc., New York, N. Y., 1950, pp. 237–243.

(14) E. Fischer, *Ber.*, **34**, 442 (1901).

(15) *Cf.* A. Colombano, G. Sanna, and I. Delitala, *Gazz. chim. ital.*, **44**, I, 97 (1914), who reported $[\alpha]^{28}D$ + 11.26° (26.4% in ethanol) for L-alanine ethyl ester, and ref. 14, p. 500, where observations similar to ours are reported. See also F. B. Kipping and W. J. Pope, *J. Chem. Soc.*, 494 (1926).

tion as described gave 4.7 g. (63%) of D-IIb, b.p. 66–67.5°, $n^{18}D$ 1.4139, $[\alpha]^{20}D$ + 8.1° (0.209 g./10 ml. in absolute ethanol); lit.[11] b.p. 66–67°, $n^{27.5}D$ 1.4156 (for D,L-IIb), $[\alpha]^{25}D$ −12.4° (0.258 g./10 ml. in ethanol).[16] L-IIb (3.6 g. obtained in 60% yield) had b.p. 66–67.5°, $n^{18}D$ 1.4132, $[\alpha]^{31}D$ −9.4° (0.203 g./10 ml. in absolute ethanol).

D-(−)-VI and L-(+)-VI.—A solution of 3.4 g. (0.06 mole) of D-IIb in 3.0 ml. of water was added dropwise to a cold (15°), stirred mixture of 2.15 g. (0.03 mole) of III and 3.0 ml. of water. When the addition was complete, the mixture was allowed to stand for 48 hr. before distillation. D-(−)-VI (2.06 g., 54%) had b.p. 59–59.5° (2 mm.), $n^{20}D$ 1.4623, $[\alpha]^{20}D$ −40.0° (0.113 g./10 ml. in absolute ethanol). Similar treatment of a mixture of 1.75 g. (0.024 mole) of III and 2.0 ml. of water with a solution of 2.85 g. (0.05 mole) of L-IIb and 2.0 ml. of water yielded 1.43 g. (45%) of L-(+)-VI, b.p. 60–61° (2 mm.), $n^{21}D$ 1.4623, $[\alpha]^{20}D$ 40.0°.

The infrared spectra of VI, D-(−)-VI, and L-(+)-VI were virtually superimposable, as were the n.m.r. spectra of VI and L-(+)-VI.

Nuclear Magnetic Resonance Spectra.—The n.m.r. spectra of VI and VII contained a series of lines from τ = 3.9 to 5.3. This region is characteristic of vinyl and hydroxyl resonances, and since sets of lines similar to these low-frequency patterns have been noted in the simpler spectrum of Id,[5] these lines are assigned to the vinyl and hydroxyl hydrogens of Ib, Ic, IVb, and IVc. No attempt was made to analyze the vinyl hydrogen spectrum of Ib or IVb. The spectrum of each of the products contained a quartet, $J \approx 6.2$, centered about $\tau = 5.96 \pm 0.04$. The resonance frequency and intensity indicated that this band was due to the allylic hydrogen of the 1-(1-aziridinyl)-3-buten-2-ol (I), and this was confirmed by fractionation of VI and VIII. The quartet pattern indicates that the coupling constants of the allylic hydrogen with the C_1- and C_3-hydrogens are about equal. The bands centered about τ = 6.55 have been noted earlier in this paper. The spectra of VI and VII had a number of lines from τ = 7.5 to 8.9 and 7.4 to 9.0, respectively. These high frequency lines are assigned to the C_1-hydrogens of the corresponding 1-(1-aziridinyl)-3-buten-2-ol (I), the C_2-hydrogens of the corresponding 2-(1-aziridinyl) 3-buten-1-ol (IV), and the ring hydrogens and methyl hydrogens of I and IV.[3,5] The bands of the C_1-hydrogens of I, doublets centered at $\tau = 7.75 \pm 0.05$, overlap a portion of the bands due to the C_2-hydrogen of IV. The complex patterns of the ring hydrogens of Ib and IVb (and IIb) appear at τ = 8.0 to 8.6, and the C-methyl resonances appear at highest field. The spectrum of the highest boiling fraction obtained by distillation of VI had a band at τ = 5.9, which was of greater intensity than expected from the intensity of the band at τ = 7.75, and a band at τ = 7.1. If the band at τ = 7.1 and a portion of the band at τ = 5.9 are due to VIII, then the highest boiling fraction consisted of 85–90% IVb, 3–5% Ib, and 6–10% VIII.

VI was analyzed as follows: letting x equal the area of the band at τ = 6.55 due to the C_1-hydrogens of IVb, y equal the area of the bands due to the vinyl and hydroxyl hydrogens of Ib and IVb at τ = 3.9 to 5.3, and z equal the area of the bands from τ = 7.5 to 8.9 due to the C_1-hydrogens of Ib, the C_2-hydrogens of IVb, and the ring hydrogens and methyl hydrogens of Ib and IVb, $N = 2x/y \backsimeq 4x/z$, where N is the mole fraction of IVb. Because of the complex nature of the spectra and the lack of a base line stabilizer, estimates of product compositions are considered accurate to only ±5%.

(16) Freshly distilled D,L-IIb obtained from Monomer-Polymer Corp. had $n^{22.5}D$ 1.4112. Poly-D-propylenimine has a much more negative specific rotation than L-IIb.[11]

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Structure–Activity Relationships of 1-[(3-Indolyl)alkyl]-4-arylpiperazines. A New Series of Tranquilizers

BY DAVID W. WYLIE AND S. ARCHER

Departments of Pharmacology and Chemistry, Sterling-Winthrop Research Institute, Rensselaer, New York, U.S.A.

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Compounds of a new series of indolylalkylphenylpiperazines have been found active as central nervous system depressants; in general they exhibited properties similar to those of other agents used in the treatment of psychoses. Certain relationships exist between structure and activity. Although these agents possess peripheral adrenolytic activity to the same degree as the phenothiazines and benzodioxanes, no definite correlation could be found between various central activities and peripheral adrenolytic activity.

Various members of a new series of indolylalkylphenylpiperazines synthesized in our laboratories[1] have been found to possess potent central depressant activity. The pharmacological properties of individual compounds differ to some degree, but these properties place this series in the category of major tranquilizers. A large number of these agents has been tested and the relationship between structure and activity studied. Like other groups of potent tranquilizers, the compounds in this series possess some adrenergic blocking activity and the large number of agents tested has allowed a study of the relationship between this and central depressant activity.

Methods.—Potentiation of hexobarbital anesthesia was determined by the method previously described.[2] Male mice weighing 18 to 24 g. were used and at least three dose levels of each drug were tested using 10 mice per dose. The mice were pretreated with the compound orally and 40 min. later a subhypnotic dose of

(1) S. Archer, D. W. Wylie, L. S. Harris, T. R. Lewis, J. W. Schulenberg, M. R. Bell, R. K. Kullnig, and A. Arnold, *J. Am. Chem. Soc.*, in press.

(2) D. W. Wylie, *Proc. Soc. Exptl. Biol. Med.*, **98**, 716 (1958).